



**IN THE CLAIMS**

This listing of claims replaces all prior versions, and listings, in this application.

Claims 1-2 (canceled)

3. (currently amended) An isolated conjugate complex comprising at least one ubiquitin or a derivative thereof, and a protein; wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, and fragments and derivatives thereof, wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins; and said conjugate complex is formed via N-end rule ubiquitylation.

4. (currently amended) The conjugate complex of claim 3, wherein said conjugate complex is immobilized on a support and/or linked to a label.

5. (withdrawn and currently amended) A method for producing a conjugate complex comprising at least one ubiquitin or a derivative thereof, and a protein; wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6, and fragments and derivatives thereof, wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins, comprising:

- a) forming a mixture comprising a vector containing a clone coding for said protein, an in vitro transcription/translation system, an N-rule ubiquitylation system and, optionally, a proteasome inhibitor; and
- b) incubating said mixture to allow production of said conjugate complex.

6. (withdrawn and currently amended) The method of claim 5, further comprising:

- c) isolating said conjugate complex.

7. (withdrawn) The method of claim 6, wherein said isolating is done by binding to an antibody specific to a poly-ubiquitin chain.

8. (withdrawn) The method of claim 6, wherein said isolating is done by binding to an antibody specific for said protein.

Claim 9 (canceled)

10. (withdrawn) An isolated activated fragment of a protein, said fragment having an exposed N-degron, wherein said protein has a hidden N-degron and is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof.

Claim 11 (canceled)

12. (withdrawn) The activated fragment of claim 10, wherein said activated fragment is immobilized on a support and/or linked to a label.

Claims 13-14 (canceled)

15. (withdrawn) A method of producing an activated fragment of a protein having an exposed N-degron, wherein said protein is selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6 and fragments and derivatives thereof, comprising:

- a) forming a mixture comprising said protein and a protease which cleaves said protein to form said activated fragment; and
- b) incubating said mixture to allow production of said activated fragment.

16. (withdrawn) The method of claim 15, further comprising:

c) isolating said activated fragment.

17. (currently amended) A composition comprising a conjugate ~~complex~~ comprised of at least one ubiquitin and a protein, wherein said protein is selected from the group consisting of aprataxin, SLP, HMG17, PinX1, CIR, HMGN3, HSPC144, Cullin 3, CDC6 and fragments and derivatives thereof, wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins; said conjugate ~~complex~~ is formed via N-end rule ubiquitylation; and said conjugate ~~complex~~ is immobilized on a support and/or linked to a label.

Claims 18-34 (canceled)

35. (withdrawn) A method for identifying one or more active compounds that modulate N-end rule dependent ubiquitylation of a protein selected from the group comprising aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6, comprising:

- a) forming a mixture comprising said protein or a fragment or derivative thereof, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;
- b) measuring N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein or a fragment or derivative thereof; and
- c) identifying one or more compounds that modulate the rate of ubiquitylation or degradation.

36. (withdrawn) The method of claim 35, wherein said protein or a fragment or derivative thereof includes a pro-N-degron and said mixture of step a) further includes a protease which exposes said N-degron.

37. (withdrawn) The method of claim 35, wherein said protein or a fragment or derivative thereof is an activated fragment of said protein having an exposed N-degron.

38. (withdrawn) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase, E3 ligase, a protease that exposes said N-degron, or a combination thereof.

39. (withdrawn) The method of claim 35, wherein said active compound modulates activity of an E1 ligase, E2 ligase and/or E3 ligase, or a combination thereof.

40. (withdrawn) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

- a) performing the identifying method of claim 36;
- b) repeating said identifying method, except that said mixture further comprises an inhibitor of N-end rule ubiquitylation or said protein is replaced with a pre-activated fragment of said protein having said exposed N-degron; and
- c) determining whether said compound is specific for said protease, and/or said N-end rule ubiquitylation system.

41. (withdrawn) A method for determining the mechanism of a compound that affects N-end rule ubiquitylation, comprising:

- a) performing the identifying method of claim 35;
- b) repeating said identifying method, except that said mixture further comprises an additional modulator of Type I, Type II and/or Type III N-end rule ubiquitylation; and
- c) determining where said compound affects Type I, Type II and/or Type III N-end rule ubiquitylation.

42. (withdrawn) A method of making a pharmaceutical formulation containing one or more active compounds which modulate N-end rule ubiquitylation of a protein selected

from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMG13, HSPC144 and CDC6, comprising:

- a) forming a mixture comprising said protein, or an activated fragment of said protein having an exposed N-degron, an N-rule ubiquitylation system, one or more candidate compounds and, optionally, a proteosome system;
- b) detecting N-end rule ubiquitylation and/or proteosome-mediated degradation of said protein;
- c) identifying one or more active compounds from said one or more candidate compounds; and
- d) incorporating at least one of said one or more active compounds into a pharmaceutical formulation comprising said at least one active compound and suitable carrier.

43. (withdrawn) The method of claim 42, wherein said one or more active compounds are inhibitors of N-end rule ubiquitylation.

44. (withdrawn) The method of claim 42, wherein said one or more active compounds are promoters of N-end rule ubiquitylation.

45. (withdrawn) The method of claim 42, wherein said one or more active compounds are naturally occurring.

46. (withdrawn) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library.

47. (withdrawn) The method of claim 42, wherein said one or more candidate compounds are selected from a compound library of FDA approved drugs.

Claims 48-54 (canceled)

55. (withdrawn) A library of N-end rule ubiquitylation substrates including at least two proteins, or fragments or derivatives thereof, selected from the group consisting of aprataxin, tau, SLP, HMG17, PinX1, CIR, Cullin 3, HMGN3, HSPC144 and CDC6.

Claim 56 (canceled)

57. (currently amended) An isolated conjugate complex comprising at least one ubiquitin or a derivative thereof, and a recombinant protein; wherein said recombinant protein is selected from the group consisting of tau, and fragments and derivatives thereof, wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins; and said conjugate complex is formed via N-end rule ubiquitylation.

58. (currently amended) The conjugate complex of claim 57, wherein said conjugate complex is immobilized on a support and/or linked to a label.

59. (currently amended) A composition comprising a conjugate complex comprised of at least one ubiquitin and a protein, wherein said protein is selected from the group consisting of tau, and fragments and derivatives thereof, wherein said fragments and derivatives thereof comprise polypeptides of at least 50 amino acids having at least 90% sequence identity to sequences within their corresponding proteins; said conjugate complex is formed via N-end rule ubiquitylation; and said conjugate complex is immobilized on a support and/or linked to a label.

60. (currently amended) The conjugate complex of claim 3, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

61. (currently amended) The conjugate complex of claim 60, wherein said activated fragment is immobilized on a support and/or linked to a label.

62. (currently amended) The conjugate ~~complex~~ of claim 57, wherein said fragment is an activated fragment of a protein, said fragment having an exposed N-degron.

63. (currently amended) The conjugate ~~complex~~ of claim 62, wherein said activated fragment is immobilized on a support and/or linked to a label.